Translation

PCT Application
PCT/JP2002/002265

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference TR001PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/JP2002/002265	International filing date (*day/month/year*) 11 March 2002 (11.03.2002)	Priority date (*day/month/year*)
International Patent Classification (IPC) or national classification and IPC C12N 15/09, C07K 14/475, C12N 5/10, A61K 38/17, A61P 43/00, A61K 35/14, 35/12		
Applicant	REPROCELL INC.	

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 27 January 2003 (27.01.2003)	Date of completion of this report 16 January 2004 (16.01.2004)
Name and mailing address of the IPEA/JP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP2002/002265

I. Basis of the report

1. With regard to the elements of the international application:*

☒ the international application as originally filed

☐ the description:

pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:

pages _____, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:

pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:

pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP2002/002265

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 48-61, 75-85

because:

☒ the said international application, or the said claims Nos. 48-61, 75-85 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See supplemental sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 48-61, 75-85 .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: III. 1.

Claims 48-61 and 75-85 pertain to diagnostic methods or methods for treatment of the human body by therapy, and thus relate to subject matter which does not require international preliminary examination by this International Preliminary Examining Authority, under the provisions of PCT Article 34(4)(a)(i) and PCT Rule 67.1(iv).

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP2002/002265

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

See supplemental sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-47, 62-74 .

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV. 3.

The inventions set forth in claims 1-7 relate to polypeptides having a WIF domain. The polypeptide WIF-1 having a WIF domain, which has the same amino acid sequence as the amino acid sequence presented in SEQ ID NO: 4 in the present application, is known. (Nature (1999), Vol. 398, pp. 431-436)

The inventions set forth in claims 8-47 and 62-74 relate to pluripotency-maintaining stem cell compositions containing a polypeptide having a WIF domain, and to a method for maintaining the pluripotency of stem cells by using a polypeptide having a WIF domain.

The inventions set forth in claims 13-16 relate to pluripotent stem cells which do not differentiate *in vitro*. "Pluripotent stem cells which do not differentiate *in vitro*" include embryonic stem cells and precultured haematopoietic stem cells. Embryonic stem cells are known to be pluripotent. The fact that haematopoietic stem cells are pluripotent was also known before the filing date of the present application (Jikken Igaku, Vol. 19, No. 15, pp. 1977-1981)

Therefore, there is no special technical feature in the sense of PCT Rule 13.2 that is common to all of the claims, and the inventions set forth in claims 1-47 and 62-74 can be considered to comprise three groups of inventions - the inventions set forth in claims 1-7, the inventions set forth in claims 13-16, and the inventions set forth in claims 8-12, 17-47 and 62-74.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV. 3.

The International Preliminary Examining Authority considers the following claims to satisfy the condition of unity of invention.

Claims 8-12, 17-47 and 62-74

The parts of the international application considered by the International Preliminary Examining Authority to relate to the principal invention are as follows.

Claims 8-12, 17-47 and 62-74

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	8-12, 17-47, 62-74	YES
	Claims	1-7, 13-16	NO
Inventive step (IS)	Claims	8-12, 17-47, 62-74	YES
	Claims	1-7, 13-16	NO
Industrial applicability (IA)	Claims	1-47, 62-74	YES
	Claims		NO

2. Citations and explanations

Document 1: J. C. Hsieh *et al.*, "A new secreted protein that binds to Wnt proteins and inhibits their activities", Nature (1999), Vol. 398, pp. 431-436

Document 2: WO 00/05374 A2 (Incyte Pharm Inc.), 3 February 2000; entire text

Document 3: M. Drouet *et al.*, "Cell cycle activation of peripheral blood stem and progenitor cells expanded ex vivo with SCF, FLT-3 ligand, TPO, and IL-3 results in accelerated granulocyte recovery in a baboon model of autologous transplantation but G_0/G_1 and $S/G_2/M$ graft cell content does not correlate with transplantability", Stem Cells (2001), Vol. 19, No. 5, pp. 436-442

Document 4: M. Drouet *et al.*, "The reduction of in vitro radiation-induced Fas-related apoptosis in $CD34^+$ progenitor cells by SCF, FLT-3 ligand, TPO, and IL-3 in combination resulted in $CD34^+$ cell proliferation and differentiation", Stem Cells (1999), Vol. 17, No. 5, pp. 273-285

Document 5: Hideo Ema *et al.*, "Zouketsu kansaibo no junka to 'clonal'-na kaiseki", Jikken Igaku (2001),

Vol. 19, No. 15, pp. 1977-1981

Document 6: M. Lako *et al.*, "Characterization of Wnt gene expression during the differentiation of murine embryonic stem cells in vitro: role of Wtn3 in enhancing haematopoietic differentiation", Mech. Dev. (2001), Vol. 103, No. 1-2, pp. 49-59

Document 7: C. Brandon *et al.*, WNT signalling modulates the diversification of hematopoietic cells", Blood (2000), Vol. 96, No. 13, pp. 4132-4141

Document 8: D. J. Van den Berg *et al.*, "Role of members of the Wnt gene family in human hematopoiesis", Blood (1998), Vol. 92, No. 9, pp. 3189-3202

The inventions set forth in claims 1-7 are not novel and do not involve an inventive step in the light of documents 1 and 2, cited in the international search report.

Documents 1 and 2 disclose the polypeptide WIF-1, and cloning of a gene coding WIF-1.

The inventions set forth in claims 13-16 are not novel and do not involve an inventive step in the light of document 5, cited in the international search report.

Document 5 discloses haematopoietic stem cells.

The inventions set forth in claims 8-12, 17-47 and 62-74 do not involve an inventive step in the light of documents 1-5, cited in the international search report, and subsequently discovered documents 6-8.

Documents 1 and 2 disclose the polypeptide WIF-1, and cloning of a gene coding WIF-1. Document 1 in particular discloses the fact that polypeptide WIF-1 binds to Wnt proteins, and acts to inhibit the morphogenetic

signal transmitting activity of Wnt proteins.

The inventions set forth in claims 8-12, 17-47 and 62-74 differ from the inventions disclosed in documents 1 and 2 in that the former are compositions for stem cell survival in which a polypeptide having a WIF domain is used, whereas the latter disclose the use of polypeptide WIF-1 in such compositions.

However, subsequently discovered documents 6-8 indicate that Wnt polypeptides have a signalling function in the differentiation of stem cells into blood cells

Maintaining the pluripotency of stem cells such as haematopoietic stem cells in the undifferentiated state had been widely attempted at the time of filing the present application and was a problem which was natural for a person skilled in the art to consider. Therefore, from the disclosures in documents 6-8 a person skilled in the art could easily investigate addition of the WIF-1 in the inventions disclosed in documents 1 and 2 to a medium for culturing haematopoietic stem cells, as an inhibitor of the capabilities of Wnt polypeptides in relation to differentiation and development regarding morphogenesis or differentiation into blood cells, in order to maintain the haematopoietic stem cells in the undifferentiated state, and attempt to use WIF-1 in medicinal compositions for the same purpose.

Addition of stem cell survival factors SCF and/or FLT-3 ligand to the medium for stem cell survival also does not involve an inventive step, since this is disclosed in documents 3-5, 7 and 8, etc.